

NOTES

Recovery of *Herbaspirillum* Species from Persons with Cystic Fibrosis[▽]

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***Herbaspirillum* species are not known to be human pathogens. We report on the identification of *Herbaspirillum* from cultures from 28 persons with cystic fibrosis (CF). Most isolates were initially identified as members of the *Burkholderia cepacia* complex. Although the role that these species play in lung disease in persons with CF is not known, their differentiation from other species is important and has serious implications for clinical care and patient well-being.**

Cystic fibrosis (CF), which results from mutations in the cystic fibrosis transmembrane conductance regulator, is characterized by multiorgan dysfunction and a predisposition to chronic infection of the respiratory tract. Although bacteria pathogenic for humans, such as *Staphylococcus aureus* and *Haemophilus influenzae*, are important in respiratory tract infections in persons with CF, particularly among young patients, a variety of opportunistic human pathogens also play a role in lung disease in persons with CF. Among these, *Pseudomonas aeruginosa* is the most important, infecting ~80% of adult patients with CF. Other species, such as members of the *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans*, are less commonly found but may be associated with accelerated lung disease and poor outcomes in persons with CF. Advances in bacterial taxonomy and genetics-based identification methodologies have led to a growing appreciation that a variety of other species may also be recovered from the respiratory tract of persons with CF.

Since January 2000, we have identified *Herbaspirillum* species in cultures of specimens obtained from multiple CF patients, including a recent isolate recovered from a culture of blood. We present the latter case, describe our experience with identifying *Herbaspirillum* from other CF patients, and discuss the implications of the correct identification of these species in this patient population.

Case report. A 26-year-old man with CF characterized by moderate to severe lung disease, pancreatic insufficiency, diabetes, and liver disease had been stable until 2006, when he required multiple hospital admissions for exacerbations of respiratory symptoms. He received several courses of intrave-

nous antibiotics, but the recovery of pulmonary function became progressively less, as noted on spirometry, until he ultimately required 1 liter of supplemental oxygen via a nasal cannula at night. He was admitted to the hospital in late 2007 for a mild exacerbation of lung disease. A culture of sputum was positive for methicillin-resistant *Staphylococcus aureus* and mucoid *Pseudomonas aeruginosa*, for which he was treated with intravenous vancomycin, piperacillin-tazobactam, and tobramycin. His forced expiratory volume in the first second improved, and antibiotics were discontinued after 20 days. However, on hospital day 23, he developed fevers and rigors, and two sets of aerobic cultures of blood drawn on consecutive days were positive for a gram-negative rod, initially identified as belonging to the *Burkholderia cepacia* complex. On the basis of in vitro susceptibility testing of the recovered bacteria, the antibiotic regimen was changed to intravenous ceftazidime and tobramycin and oral trimethoprim-sulfamethoxazole (TMP-SMX), levofloxacin, and minocycline. His catheter (Port-A-Cath) was removed, he defervesced, and repeat cultures of blood and sputum samples were negative. After 4 weeks of treatment with multiple antibiotics, he was discharged to home to complete a prolonged course of oral TMP-SMX. Analysis of the complete 16S rRNA gene sequences of the blood culture isolates recovered demonstrated a similarity level of 99.9% with sequences of *Herbaspirillum* species found in the NCBI database (<http://www.ncbi.nlm.nih.gov>); the level of similarity to *Herbaspirillum putei* type strain ATCC BAA-806 was 99.6%.

Between January 2000 and December 2007, the *Burkholderia cepacia* Research Laboratory and Repository (BcRLR; University of Michigan, Ann Arbor) identified *Herbaspirillum* species from among the isolates recovered from 28 CF patients, including the patient described above. The isolates had been referred for evaluation from 23 CF treatment centers in the United States. The referring laboratories had used a variety of commercial test systems in their initial evaluation of these isolates, which identified 19 (68%) of 28 of the isolates as members of the *Burkholderia cepacia* complex; 4 (14%) were

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TABLE 1. Putative species identification and phenotypic test systems used by referring laboratories and growth of *Herbaspirillum* isolates on BCSA

Species identified	No. of isolates identified by:							Growth on BCSA		
	Phenotypic test system									
	MicroScan ^a	Rapid NF ^b	Vitek ^c	API NF ^c	Phoenix ^d	Biochem ^e	NA ^f	+	+/-	-
<i>Burkholderia</i> (n = 19)	2	10	3	1	1	2		11	4	4
<i>Ralstonia</i> (n = 4)	2		1				1	2	1	1
NLFGNR ^g (n = 5)	1		3	1				2	2	1

^a Siemens Medical Solutions Diagnostics (Dade Behring Inc.), Deerfield, IL.

^b Remel Inc., Norcross, GA.

^c bioMérieux, Durham, NC.

^d BD Biosciences, Franklin Lakes, NJ.

^e Biochem, short biochemical battery.

^f NA, method not available.

^g NLFGNR, non-lactose-fermenting gram-negative rod.

identified as *Ralstonia* species, and the remaining 5 (18%) were not identified to the species level (Table 1).

Evaluation of the isolates at BcRLR showed variable growth on *B. cepacia* selective agar (BCSA [4]), with most (78%) showing good to moderate growth on this selective medium (Table 1). All isolates were grown aerobically on Mueller-Hinton broth (Becton Dickinson, Cockeysville, MD) supplemented with 1.6% (wt/vol) agar and were incubated at 32°C for 24 to 48 h. DNA was prepared from the bacterial cultures as described previously (12). A 16S rRNA-targeted PCR assay specific for all *Burkholderia*, *Ralstonia*, and *Pandora* species was performed as described previously (9); and the results were negative for all 28 isolates. Previously published 16S rRNA-directed PCR assays specific for other CF-related bacterial species, including *Pseudomonas*, *Stenotrophomonas*, and *Achromobacter* (10, 12, 14, 15), were also negative. Complete 16S rRNA-targeted PCR amplification, sequencing, and editing were performed as described previously (12). The edited and assembled sequences were compared to those available in the NCBI GenBank bacterial DNA database. The isolates were identified as *Herbaspirillum* species (i.e., to the genus level) if the identities of the 16S rRNA sequence to the 16S rRNA sequences of the *Herbaspirillum* species in the database were greater than 95%; isolates were tentatively identified to the species level if the identity was 99.5% or greater to *Herbaspirillum* reference strains available in the database, which included *Herbaspirillum rubrisubalbicans* ATCC 19308^T, *Herbaspirillum seropedicae* ATCC 35892^T, *H. putei* ATCC BAA-806^T, *Herbaspirillum huttiense* ATCC 14670^T, and *Herbaspirillum frisingense* DSM 13128^T. On the basis of that analysis, we identified three isolates as *H. huttiense*, three as *H. frisingense*, two as *H. seropedicae*, and two as *H. putei*. Eighteen isolates could not be assigned to a specific *Herbaspirillum* species; these appeared to represent at least three distinct lineages. The first lineage included 14 isolates whose 16S rRNA sequences shared >99% similarity with those of the *H. huttiense* and *H. putei* type strains (Fig. 1). The other two lineages each comprised a single strain and appeared to represent novel *Herbaspirillum* species. Further taxonomic work is required to elucidate the taxonomic status of all of these isolates.

Herbaspirillum species are gram-negative, motile, nitrogen-fixing bacteria belonging to the class *Betaproteobacteria* (1).

They include pathogens and endophytes of various plants, including corn, wheat, rice, sugarcane, sorghum, banana, and pineapple (1, 3, 11). The genus currently consists of 10 named species: *Herbaspirillum autotrophicum*, *Herbaspirillum chlorophenicum*, *H. frisingense*, *Herbaspirillum hiltneri*, *Herbaspirillum huttiense*, *Herbaspirillum lusitanum*, *H. putei*, *Herbaspirillum rhizosphaerae*, *H. rubrisubalbicans*, and *H. seropedicae*. These species are not known to be human pathogens. Only a single case of human infection due to *Herbaspirillum* has been described in detail (13). This involved bacteremia and cellulitis due to *H. seropedicae* in a 49-year-old homeless man with cirrhosis. *Herbaspirillum* has been reported, however, to have been recovered from various human sources, including wounds, the respiratory tract, gastric juice, feces, urine, eye and ear samples, and cases of otitis and bacteremia (2). Most of these isolates were not identified to the species level; rather, similar to most of the isolates that we report on, these appeared to occupy a novel taxon within this genus. These isolates, as well as ours and several additional *Herbaspirillum* isolates from Canada, Germany, Italy, Switzerland, and the United Kingdom, are currently undergoing comprehensive polyphasic characterization to clarify their taxonomic status.

The frequency with which *Herbaspirillum* species are involved in pulmonary infection in persons with CF is not clear. During the 8-year interval of our study, we analyzed isolates from sputum from greater than 1,100 CF patients, finding *Herbaspirillum* in only 28 patients (<3%). Although this rate of recovery is comparable to that of some other recognized CF pathogens, such as certain *B. cepacia* complex species, conclusions regarding the frequency of *Herbaspirillum* infection in persons with CF are limited in our study by bias in the sample set (i.e., care centers are more likely to refer for analysis "atypical" isolates for which species identification may be in question). We confirmed the presence of other, more common CF-related pathogens, including *Achromobacter*, *Ralstonia*, *Burkholderia*, and *Stenotrophomonas*, in only 3 (11%) of the 28 patients. This raises the possibility that the recovery of *Herbaspirillum* may be influenced by the presence of other species that might overgrow *Herbaspirillum* in mixed cultures. Again, however, a prospective study of a nonbiased sample set is necessary to draw firm conclusions regarding the frequency of *Herbaspirillum* in persons with CF.

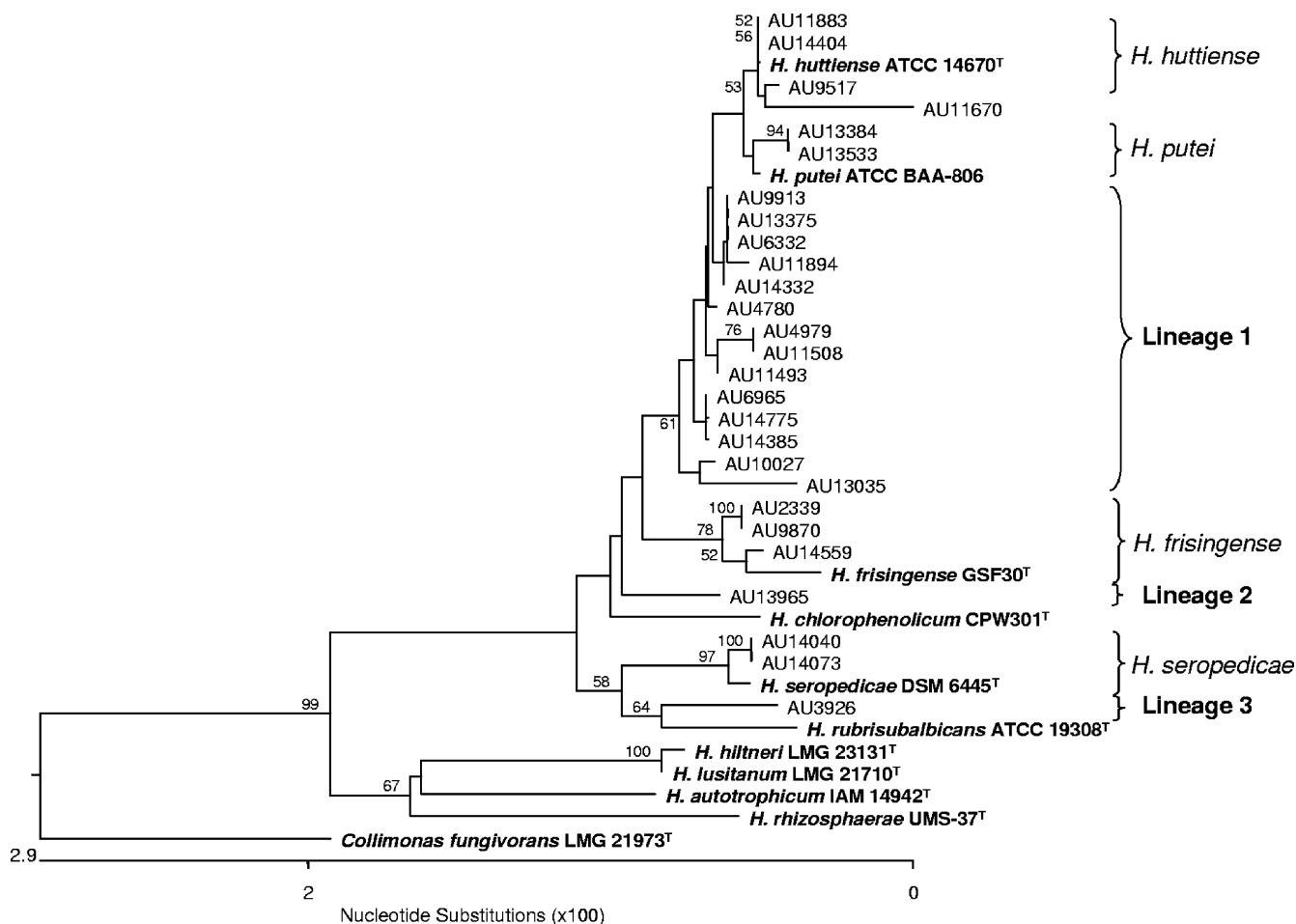


FIG. 1. Phylogenetic tree generated by using ClustalV-based alignment of the 16S rRNA sequences of isolates obtained from respiratory specimens from CF patients, as well as 11 type strains (boldface type) within the genus *Herbaspirillum*. All sequences were trimmed to approximately 1,354 bases to provide equal sample weighting and were aligned with MegAlign software (DNASStar Inc., Madison, WI). The tree is rooted with *Collimonas fungivorans* LMG 21973^T. Bootstrap values greater than 50 (based on 1,000 samplings) are shown at the branching points.

The role that *Herbaspirillum* species may play in the progression of pulmonary disease in persons with CF is also not clear. The 28 *Herbaspirillum*-infected patients ranged in age from 20 months to 59 years; the median age at the time of the positive culture was 17 years. For 27 of the 28 patients, we identified only a single *Herbaspirillum* isolate among the isolates serially obtained from cultures of sputum, suggesting transient colonization with *Herbaspirillum*. However, chronic infection is possible; in one patient, analysis of serial isolates demonstrated the presence of the same *Herbaspirillum* strain for 3 years.

Our analysis demonstrates a failure of commercially available microbial identification systems to identify *Herbaspirillum* species. None of the 28 isolates were identified as *Herbaspirillum* by the referring laboratories; 85% were initially identified as another opportunistic species more commonly found in cultures of sputum from persons with CF. Misidentification of *Herbaspirillum* as *Burkholderia* was particularly common and was likely due to the close phylogenetic and phenotypic relatedness of the species within these genera (Table 2). The failure of commercial test systems to identify *Herbaspirillum* and the similarity of the phenotypic profile of *Herbaspirillum* species

with those of other CF-related pathogens supports the use of genetics-based methods for the identification of these species.

The misidentification of *Herbaspirillum* as *Burkholderia* can have serious consequences for CF patients. Most *Burkholderia* strains exhibit constitutive and inducible resistance to the available antimicrobial agents, and infection is generally refractory to therapy (7). Strict infection control policies are recommended to segregate persons infected with *B. cepacia* complex species from other CF patients in order to reduce the risk of the interpatient spread of *Burkholderia* (6). These measures place an enormous psychosocial burden on the patient, as well as a considerable economic burden on treatment centers. The poor prognosis associated with *Burkholderia* infection is a further source of anxiety for CF patients, their families, and caregivers. Perhaps most importantly, due to the particularly poor outcomes after lung transplantation among persons infected with *Burkholderia*, many treatment centers consider *Burkholderia* infection an absolute contraindication to transplantation, thus denying patients with end-stage pulmonary disease the only option for survival (5).

TABLE 2. Characteristics of *Herbaspirillum* and other related CF-relevant species^a

Test	Result for:																	
	<i>Herbaspirillum</i> ^b	<i>Ralstonia</i>			<i>Cupriavidus</i>			<i>B. cepacia</i> complex genomovars ^c									<i>Burkholderia gladioli</i>	<i>Pandoraea</i>
		<i>R. pickettii</i>	<i>R. mannitolilytica</i>	<i>R. insidiosa</i>	<i>C. respiraculi</i>	<i>C. gilardii</i>	<i>C. paucula</i>	I	II	III	IV	V	VI	VII	VIII	IX		
Oxidase	+	(93)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	v
ONPG ^d	+	(93)	—	v	v	—	v	ND	+	+	+	v	+	+	+	v	+	v
Lysine decarboxylase	—	(0)	—	—	—	—	—	+	v	+	+	+	—	+	v	+	—	—
Acid from:																		
Lactose	v	(11)	v	+	—	—	—	+	+	+	v	+	+	+	+	+	—	—
Sucrose	—	(0)	—	—	—	—	—	v	—	+	—	+	—	+	v	v	—	—

^a The data for *Herbaspirillum* are from this study; all other data are from a previous report (8). +, >90% positive; —, >90% negative; v, variable; ND, not determined.

^b Numbers in parentheses are percent positive.

^c The genomovars of the *B. cepacia* complex are as follows: I, *B. cepacia*; II, *B. multivorans*; III, *B. cenocepacia*; IV, *B. stabilis*; V, *B. vietnamiensis*; VI, *B. dolosa*; VII, *B. ambifaria*; VIII, *B. anthina*; IX, *B. pyrrocinia*.

^d ONPG, o-nitrophenyl-β-D-galactopyranoside.

In summary, we identified *Herbaspirillum* in cultures of sputum from several CF patients. Initial phenotypic evaluation identified none as *Herbaspirillum*; differentiation from other CF-related pathogens required genetics-based analyses. Most patients appeared to have had transient respiratory tract colonization with *Herbaspirillum*; however, bacteremia in one patient and a chronic (3-year) respiratory tract infection in another were observed. Although the role that *Herbaspirillum* may play in contributing to lung diseases in persons with CF requires further study, misidentification of these species, particularly as *Burkholderia*, has serious implications for clinical care and patient well-being.

Nucleotide sequence accession numbers. All 16S rRNA gene sequences determined in this study were deposited in the NCBI GenBank database under accession numbers EU549832 through EU549859.

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None of the authors has financial interests to declare.

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